

## Midterm 2 Solutions

1) A Berkeley undergrad was working in a biochem lab and needed to make a solution that was exactly pH=6.3. She took her stock buffer solution to the pH meter with acid and base so she could adjust the pH, but was disappointed when he found the pH meter was broken. However she was taking Chem 130A and remembered the prof saying that a pH meter was just an electrochemical cell. She also remembered that a grad student had shown her a fancy new digital voltmeter in the lab. She took the pH probe (the actual electrochemical cell part) off of the meter, hooked it to the leads of the voltmeter and read 0.0208 V when she put the probe into a pH 7.00 standard solution. She switched to a pH 5.00 standard, and read 0.1392 V.

$$T = 298$$

What should the voltage read when her solution is the desired pH=6.3?

$$E = E^{\circ} - \frac{0.0591}{n} \log Q$$

$$E = E^{\circ} - \frac{RT}{nF} \ln Q \quad Q = K [H^+]^x$$

$$\Delta E (\text{pH } 7 - \text{pH } 5) = -0.1184 \text{ V} = -0.0591 \cdot x - \log \frac{10^{-7}}{10^{-5}}$$

$$x = -1$$

$$\begin{aligned} \Delta E (\text{pH } 7 - \text{pH } 6.3) &= 0.0208 + 0.0591 \cdot (7 - 6.3) \\ &= 0.06217 \text{ Volts} \end{aligned}$$

$$\Delta E = -\frac{RT}{nF} \ln Q(\text{pH } 7) + \frac{RT}{nF} \ln Q(\text{pH } 5)$$

$$= -\frac{RT}{nF} \ln \frac{[10^{-7}]^x}{[10^{-5}]^x} \quad \text{all else cancels}$$

2) A grad student was working on a regulatory protein, which she believed was deactivated through proteolysis in the cell. She thought that the proteolysis would occur only in the unfolded state of the protein. To test this she wanted to destabilize the protein, but not cause it to totally unfold. She remembered that hydrophobic amino acids are usually buried, and hydrophilic ones are not. The structure of the protein had been determined and she decided to convert a buried valine residue to ~~aspartic~~ glutamic acid by introducing a mutation in the gene. To estimate whether this effect would be of the right size she looked at reference data on partitioning of the amino acids between octanol and water (two solvents which do not mix, octanol being fairly hydrophobic and water being hydrophilic). In this measurement the amino acid is added to a mixture of the two solvents, shaken until the system comes to equilibrium, and then the concentration of amino acid in each phase is measured. The partition factor  $P$  is the concentration in water divided by the concentration in octanol. She realized this was closely related to using solubilities, which she had learned about before. For the wild type amino acid valine she found  $P=0.06033$  from the table, and for "mutant" glutamic acid she found  $P=4.383$ .

Use these data to estimate the change in free energy of folding of the protein upon introduction of the mutation relative to the wild type sequence protein.

$$P = \text{eqil constant}, \quad \Delta G_{\text{trans}}^{\circ} = -RT \ln P$$

$$\Delta G_{\text{val}} = -8.341 \cdot 298 \cdot \ln(0.06033) = +6.96 \text{ kJ/mol}$$

$$\Delta G_{\text{glu}} = -8.341 \cdot 298 \cdot \ln(4.383) = -2.48 \text{ kJ/mol}$$

Val was favorable, Glu now unfav.

$$\Delta \Delta G = \Delta G_{\text{val}} - \Delta G_{\text{glu}} = 9.44 \text{ kJ/mol}$$

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If the  $\Delta G_{\text{folding}}$  for the wild type protein was  $-43 \text{ kJ/mol}$  at  $25^{\circ}\text{C}$ , calculate the fraction of the protein which would be unfolded for the mutant sequence.

$$\text{new } \Delta G = -33.56 \text{ kJ/mol}$$

$$K_{\text{eq}} = \frac{f}{u} = e^{-\Delta G^{\circ}/RT} = 7.63 \times 10^5$$

$$\text{fract unfolded} = \frac{[u]}{[u]+[f]} \approx \frac{[u]}{[f]} = \frac{1}{K} = 1.3 \times 10^{-6}$$

3) A researcher isolated a protein by adding a salt that can precipitate proteins, ammonium sulfate  $(\text{NH}_4)_2\text{SO}_4$ , molec weight 132 g/mol. His protein did precipitate when he added fairly concentrated ammonium sulfate, and he collected the precipitate and then dried it. He weighed the dried material and found he had 10 mg ( $10 \times 10^{-3}$  g) and was happy until he realized there was probably a lot of salt from liquid that had been around the precipitate he collected. Fortunately the lab had an osmometer, for measuring osmotic pressure and he decided he could measure how much protein he really had.

He dissolved his 10 mg sample in 1 ml of water, and using a membrane that would let salt through but not the protein he determined an osmotic pressure of 0.00240 atm at 293K. He knew his protein should be about 10,000 g/mol molecular weight. How much of his sample was protein, and how much was salt?

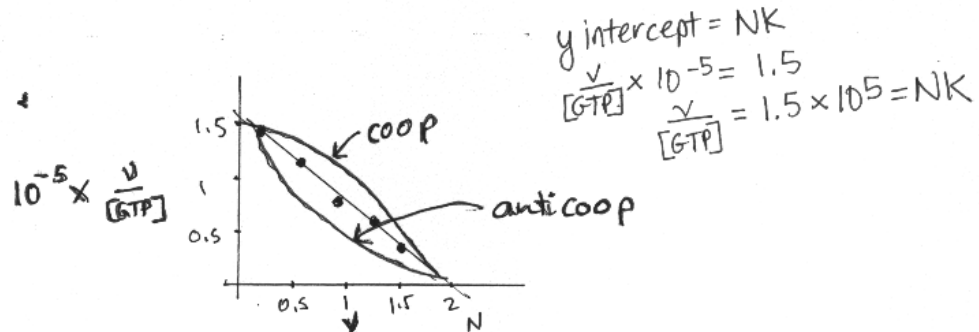
$$\pi = CRT \quad C = \frac{\pi}{RT} = \frac{0.00240 \text{ atm}}{0.08205 \cdot 293 \frac{\text{L} \cdot \text{atm}}{\text{mol}}}$$

$$= 10^{-4} \frac{\text{mol}}{\text{L}}$$

$$10^{-4} \frac{\text{mol}}{\text{L}} \cdot 10^4 \frac{\text{g}}{\text{mol}} = 1 \frac{\text{g}}{\text{L}} = 1 \frac{\text{mg}}{\text{ml}}$$

.. of 10 mg just 1 mg was protein  
9 mg was salt.

4) The researcher in problem 3 then carried out measurements of the binding of GTP to his protein. He determined the number of molecules bound per protein,  $v$ , as a function of the concentration of GTP, using equilibrium dialysis and a ~~table~~ generated the data shown in the curve below.



What are the number of molecules binding per protein and the equilibrium constant for binding?

$$N = 2$$

$$N \cdot K = 1.5 \times 10^5$$

$$K = 7.5 \times 10^4$$

Is the binding of <sup>GTP</sup> to this protein cooperative, anticooperative or independent? Sketch and label on the graph above what the curves might look like for the two choices you thought were incorrect.

straight line so independent.